

10/030351

Date of Deposit <u>January 7 2002</u>		Certificate of Mailing	Label Number <u>ELT14747622US</u>
I hereby certify under 37 C.F.R. § 1.10 that this correspondence is being deposited with the United States Postal Service as "Express Mail Post Office to Addressee" with sufficient postage on the date indicated above and is addressed to BOX PCT, Assistant Commissioner for Patents, Washington, D.C. 20231			
Guy E. Beardsley		<i>Guy E. Beardsley</i>	
Printed name of person mailing correspondence		Signature of person mailing correspondence	
Substitute Form PTO 1390 U.S. Department of Commerce Patent and Trademark Office		Attorney's Docket Number. 06727/008002	
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U.S. Application Number: Not known yet	
INTERNATIONAL APPLICATION NUMBER	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED	
PCT/US00/00264	06 January 2000	06 January 1999	
TITLE OF INVENTION	EXPRESSION OF SECRETED HUMAN ALPHA-FETOPROTEIN IN TRANSGENIC ANIMALS		
APPLICANTS FOR DO/EO/US	Stace Lindsay, Robert Mulroy, and Daniel Semeniuk		
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information			
1	<input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. § 371.		
2	<input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. § 371.		
3	<input checked="" type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. § 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. § 371(b) and PCT Articles 22 and 39(1).		
4	<input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19 th month from the earliest claimed priority date		
5	<input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. § 371(c)(2)) <input checked="" type="checkbox"/> a is transmitted herewith (required only if not transmitted by the International Bureau) <input type="checkbox"/> b. has been transmitted by the International Bureau <input type="checkbox"/> c. is not required, as the application was filed with the United States Receiving Office (RO/US).		
6	<input type="checkbox"/> A translation of the International Application into English (35 U.S.C. § 371(c)(2))		
7	Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. § 371(c)(3)). <input type="checkbox"/> a are transmitted herewith (required only if not transmitted by the International Bureau). <input type="checkbox"/> b. have been transmitted by the International Bureau. <input type="checkbox"/> c. have not been made, however, the time limit for making such amendments has NOT expired <input checked="" type="checkbox"/> d. have not been made and will not be made		
8	<input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. § 371(c)(3))		
9	<input checked="" type="checkbox"/> An oath or declaration of the inventors (35 U.S.C. § 371(c)(4)).		
10	<input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. § 371(c)(5))		
11	<input type="checkbox"/> An Information Disclosure Statement under 37 C.F.R. §§ 1.97 and 1.98.		
12	<input type="checkbox"/> An assignment for recording. A separate cover sheet in compliance with 37 §§ 3.28 and 3.31 is included.		
13	<input type="checkbox"/> A FIRST preliminary amendment <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment		
14	<input type="checkbox"/> A substitute specification.		
15	<input type="checkbox"/> A change of power of attorney and/or address letter.		
16	<input checked="" type="checkbox"/> Other items or information. Petition to Revive Application Under 37 C.F.R. § 1.137 (b), Sequence Statement Under 37 C.F.R. § 1.8121, and Sequence Listing		

17	The following fees are submitted: BASIC NATIONAL FEE (37 C.F.R. § 1.492(A)(1)-(5)): Neither international preliminary examination fee (37 C.F.R. § 1.482) nor international search fee (37 C.F.R. § 1.455(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO \$ 1040 00 International preliminary examination fee (37 C.F.R. § 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO \$ 890 00 International preliminary examination fee (37 C.F.R. § 1.482) not paid to USPTO but international search fee (37 C.F.R. § 1.445(a)(2)) paid to USPTO \$ 740 00 International preliminary examination fee (37 C.F.R. § 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1) - (4) \$ 710 00 International preliminary examination fee paid to USPTO (37 C.F.R. § 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) \$ 100 00	
X		
ENTER APPROPRIATE BASIC FEE AMOUNT =		\$710 00
Surcharge of \$130 for furnishing the oath or declaration later than □ 20 OR □ 30 months from the earliest claimed priority date (37 C.F.R. § 1.492(e))		\$
CLAIMS	NUMBER FILED	NUMBER EXTRA
Total claims	24 - 20 =	4
Independent claims	10 - 3 =	7
Multiple dependent claims (if applicable)		+ \$280
TOTAL OF ABOVE CALCULATIONS =		\$1,850.00
Reduction of 1/2 for filing by small entity, if applicable Applicant claims small entity status under 37 C.F.R. § 1.27		\$825 00
SUBTOTAL =		\$825 00
Processing fee of \$130.00 for furnishing the English translation later than □ 20 OR □ 30 months from the earliest claimed priority date (37 C.F.R. § 1.492(f)).		+
TOTAL NATIONAL FEE =		\$825 00
Fee for recording the enclosed assignment (37 C.F.R. 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 C.F.R. §§ 3.28, 3.31). \$40 00 per property.		+
TOTAL FEES ENCLOSED =		\$825 00
Amount to be refunded		\$
charged		\$
a. A check in the amount of \$825 00 to cover the above fees is enclosed b. Please charge my Deposit Account No. 03-2095 in the amount of \$[***] to cover the above fees. c. The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment, to Deposit Account No. 03-2095		
NOTE: Where an appropriate time limit under 37 C.F.R. §§ 1.494 or 1.495 has not been met, a petition to revive (37 C.F.R. § 1.137(a) or (b)) must be filed and granted to restore the application to pending status		
SEND ALL CORRESPONDENCE TO:		
Paul T. Clark Clark & Elbing LLP 176 Federal Street Boston, MA 02110-2214 Telephone: 617-428-0200 Facsimile: 617-428-7045		Susan M. Michaud Signature Susan M. Michaud Paul T. Clark Reg No. 30,162 Reg. No. 42,885



21559

PATENT, TRADEMARK OFFICE

10/030351
JC10 Rec'd PCT/PTO 07 JAN 2002

PATENT
ATTORNEY DOCKET NO. 06727/008002

Express Mail # : EL 74747622 VS	
Certificate of Mailing: Date of Deposit: January 7 2002	
I hereby certify under 37 C.F.R. § 1.8(a) that this correspondence is being deposited with the United States Postal Service as Express Mail Post Office to Addressee with sufficient postage on the date indicated above and is addressed to: BOX PATENT APPLICATION, Assistant Commissioner for Patents, Washington, D.C. 20231.	
Guy E. Beardsley	<i>Guy E. Beardsley</i>
Printed name of person mailing correspondence	Signature of person mailing correspondence

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Stace Lindsay et al.	Art Unit:	Not Yet Assigned
Serial No.:	Not Yet Assigned Based on PCT/US00/00264	Examiner:	Not Yet Assigned
Filed:	January 6, 2000	Customer No.:	21559
Title:	EXPRESSION OF SECRETED HUMAN ALPHA-FETOPROTEIN IN TRANSGENIC ANIMALS		

Assistant Commissioner For Patents
Washington, D.C. 20231

STATEMENT UNDER 37 C.F.R. § 1.821

As part of the patent application filed herewith, enclosed is a sequence listing in accordance with the requirements of 37 C.F.R. §§ 1.821 through 1.825 and consisting of five pages.

As required by 37 C.F.R. § 1.821(c), the sequence listing appears as a separate part of the application and is found after the Combined Declaration and Power of Attorney. Each sequence in the application appears separately in the sequence listing. And each sequence in the sequence listing is assigned a separate sequence identifier.

As required by 37 C.F.R. § 1.821(d), the sequence identifiers are used throughout the application description and claims to refer to their respective sequences.

As required by 37 C.F.R. § 1.821(e), enclosed is a diskette containing a copy of the sequence listing in computer readable form.

As required by 37 C.F.R. § 1.821(f), I hereby state that the contents of the computer readable form are the same as the contents of the paper copy.

As required by 37 C.F.R. § 1.821(g), I hereby state that this submission contains no new matter.

If there are any charges, or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: January 7, 2002

Susan M. Michaud
Paul T. Clark Susan M. Michaud
Reg. No. 30,162 Reg. No. 42,885

Clark & Elbing LLP
176 Federal Street
Boston, MA 02110
Telephone: 617-428-0200
Facsimile: 617-428-7045



21559

PATENT, TRADEMARK OFFICE



JC14 Rec'd PCT/PTO 06 MAY 2002

PCT

PATENT

ATTORNEY DOCKET NO. 06727/008002

#6

Certificate of Mailing: Date of Deposit: May 1, 2002

I hereby certify under 37 CFR 1.8(a) that this correspondence is being deposited with the United States Postal Service as **first class mail** with sufficient postage on the date indicated above and is addressed to the Assistant Commissioner of Patents, Washington, D.C. 20231.

Roselynn D. Scarfo

Printed name of person mailing correspondence

Roselynn D. Scarfo

Signature of person mailing correspondence

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Stace Lindsay et al.

Art Unit: Not Yet Assigned

Serial No.: 10/030,351

Examiner: Not Yet Assigned

Filed: January 7, 2002

Title: EXPRESSION OF SECRETED HUMAN ALPHA-FETOPROTEIN IN
TRANSGENIC ANIMALS

Assistant Commissioner for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Prior to examination, please amend the application as follows:

In the Specification:

Please insert the following:

--Cross Reference To Related Applications

This application claims priority from United States Provisional Application

60/114,995, filed January 6, 1999.--

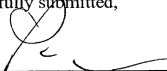
If there are any other charges, or any credits, please apply them to Deposit

Account No. 03-2095.

Date:

May 1, 2002

Respectfully submitted,



Paul T. Clark
Reg. No.30,162

Clark & Elbing LLP
101 Federal Street
Boston, MA 02110
Telephone: 617-428-0200
Facsimile: 617-428-7045



21559

PATENT TRADEMARK OFFICE

\\Ceserver\documents\06727\06727 008002 Preliminary Amendment.wpd

EXPRESSION OF SECRETED HUMAN ALPHA-FETOPROTEIN IN
TRANSGENIC ANIMALS

5

Background of the Invention

This invention relates to the expression and secretion of recombinant protein in transgenic animals.

Alpha-fetoprotein (AFP) is a 70 kDa glycoprotein produced by the
10 yolk sac and fetal liver. AFP is present in fetal serum at milligram levels, and,
at birth, declines to the nanogram levels normally found in adult serum:
increased levels of AFP in adult serum are indicative of a yolk sac tumor, a
hepatoma, or of liver regeneration. The role of AFP during fetal development
is not known, although it has been suggested that AFP may protect a gestating
15 fetus from a maternal immune attack or from the effects of maternal estrogen.

In vitro and *in vivo* experiments have shown that AFP has both cell
growth-stimulatory and -inhibitory activities, depending upon the target cell,
the relative concentration of AFP, and the presence of other cytokines and
growth factors. For example, AFP can inhibit the growth of many types of
20 tumor cells, and, in particular, inhibits estrogen-stimulated cell growth.
Conversely, AFP stimulates the growth of normal embryonal fibroblasts. AFP
has also been shown to have both immunosuppressive and immunoproliferative
effects. In order to exploit the various biological properties of AFP, it will be
necessary to obtain sufficient quantities of this molecule in an efficient and
25 cost-effective manner.

Summary of the Invention

In a first aspect, the invention features a substantially pure nucleic
acid molecule comprising: (i) a nucleic acid sequence encoding recombinant

-2-

human alpha-fetoprotein (rHuAFP), (ii) a milk-specific promoter, the promoter being operably linked to the rHuAFP-encoding sequence, and (iii) a leader sequence encoding a protein secretory signal that enables secretion of rHuAFP by milk-producing cells into the milk of a mammal.

- 5 In a second aspect, the invention features a substantially pure nucleic acid molecule comprising: (i) a nucleic acid sequence encoding recombinant human alpha-fetoprotein (rHuAFP), (ii) a urine-specific promoter, the promoter being operably linked to the rHuAFP-encoding sequence, and (iii) a leader sequence encoding a protein secretory signal that enables secretion of rHuAFP
10 by urine-producing cells into the urine of a mammal.

- In a third aspect, the invention features a non-human transgenic mammal that expresses recombinant human alpha-fetoprotein (rHuAFP) in its milk, wherein milk-producing cells of the mammal contain a transgene that comprises: (i) a nucleic acid sequence encoding rHuAFP, (ii) a milk-specific
15 promoter, the promoter being operably linked to the rHuAFP-encoding sequence, and (iii) a leader sequence encoding a protein secretory signal that enables secretion of rHuAFP by milk-producing cells into the milk of a mammal.

- In a fourth aspect, the invention features a non-human transgenic
20 mammal that expresses recombinant human alpha-fetoprotein (rHuAFP) in its urine, wherein urine-producing cells of the mammal contain a transgene that comprises: (i) a nucleic acid sequence encoding rHuAFP, (ii) a urine-specific promoter, the promoter being operably linked to the rHuAFP-encoding sequence, and (iii) a leader sequence encoding a protein secretory signal that
25 enables secretion of rHuAFP by urine-producing cells into the urine of an animal.

 In preferred embodiments of the third and fourth aspects of the invention, the mammal may be a goat, a cow, a sheep, or a pig.

-3-

In a fifth aspect, the invention features a non-human mammal's milk comprising recombinant human alpha-fetoprotein (rHuAFP). In a preferred embodiment of the fifth aspect of the invention, the rHuAFP is soluble and is produced by a non-human transgenic mammal whose milk-producing cells

5 express a transgene that comprises: (i) a nucleic acid sequence encoding rHuAFP, (ii) a milk-specific promoter, the promoter being operably linked to the rHuAFP-encoding sequence, and (iii) a leader sequence encoding a protein secretory signal that enables secretion of rHuAFP by the milk-producing cells into the milk of the mammal.

10 In a sixth aspect, the invention features a non-human mammal's urine comprising recombinant human alpha-fetoprotein (rHuAFP). In a preferred embodiment of the sixth aspect of the invention, the rHuAFP is soluble and is produced by a non-human transgenic mammal whose urine-producing cells express a transgene that comprises: (i) a nucleic acid sequence

15 encoding rHuAFP, (ii) a urine-specific promoter, the promoter being operably linked to the rHuAFP-encoding sequence, and (iii) a leader sequence encoding a protein secretory signal that enables secretion of rHuAFP by the urine-producing cells into the urine of the mammal.

In a seventh aspect, the invention features a method of producing

20 recombinant human alpha-fetoprotein (rHuAFP) that is secreted in the milk of a mammal, comprising the steps of: (a) providing a cell transfected with a transgene that comprises: (i) a nucleic acid sequence encoding rHuAFP, (ii) a milk-specific promoter, the promoter being operably linked to the rHuAFP-encoding sequence, and (iii) a leader sequence encoding a protein secretory

25 signal that enables secretion of rHuAFP by a milk-producing cell, wherein the milk-producing cell is derived from said transfected cell; (b) growing the cell to produce a mammal comprising milk-producing cells that express and secrete rHuAFP into milk; and collecting milk containing rHuAFP from the mammal.

-5-

In preferred embodiments of the tenth aspect of the invention, the rHuAFP is produced by a non-human transgenic mammal whose urine-producing cells contain a transgene that comprises: (i) a nucleic acid sequence encoding rHuAFP, (ii) a urine-specific promoter, the promoter being operably
5 linked to the rHuAFP-encoding sequence, and (iii) a leader sequence encoding a protein secretory signal that enables secretion of rHuAFP by urine-producing cells into the urine of the mammal.

In various preferred embodiments of the ninth and tenth aspects of the invention, the method may be used for the treatment of cancer, for
10 suppressing the immune system, or for inducing proliferation of bone marrow cells in a patient in need thereof.

By "human alpha-fetoprotein" or "HuAFP" or "rHuAFP" is meant a polypeptide having substantially the same amino acid sequence as the mature alpha-fetoprotein (amino acids 20-609) set forth in Genbank Accession No.
15 J00077 and encoded by the cDNA sequence set forth in Genbank Accession No. J00077 and reported in Morinaga *et al.* (*Proc. Natl. Acad. Sci. USA* 80:4604-4608, 1983).

By "human alpha-fetoprotein precursor" is meant a polypeptide having substantially the same amino acid sequence as amino acids 1-609 set
20 forth in Genbank Accession No. J00077.

By "having substantially the same amino acid sequence" is meant a polypeptide that exhibits at least 80% identity with a naturally-occurring HuAFP amino acid sequence, typically at least about 85% identity with a naturally-occurring human HuAFP sequence, more typically at least about 90%
25 identity, usually at least about 95% identity, and more usually at least about 97% identity with a naturally-occurring HuAFP sequence. The length of comparison sequences will generally be at least 16 amino acids, usually at least 20 amino acids, more usually at least 25 amino acids, typically at least 30

-6-

amino acids, and preferably more than 35 amino acids.

Sequence identity is typically measured using sequence analysis software with the default parameters specified therein, such as the introduction of gaps to achieve an optimal alignment (*e.g.*, Sequence Analysis Software

- 5 Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, WI 53705).

By "milk-producing cell" is meant a mammary epithelial cell that secretes milk.

- 10 By "urine-producing cell" is meant a bladder epithelial cell that secretes urine.

- By "promoter" is meant a minimal sequence sufficient to direct transcription. Also included in the invention are those promoter elements which are sufficient to render promoter-dependent gene expression controllable for cell type-specific, tissue-specific, temporal-specific, or inducible by external
15 signals or agents; such elements may be located in the 5' or 3' or intron sequence regions of the native gene.

- By "milk-specific promoter" is meant a promoter that naturally directs expression of a gene that is expressed in mammary epithelial cells, for example, the native promoter associated with the genes encoding whey acidic
20 protein (WAP), alpha S1-casein, alpha S2-casein, beta-casein, kappa-casein, beta-lactoglobulin, and alpha-lactalbumin.

By "urine-specific promoter" is meant a promoter that naturally directs expression of a gene that is expressed in bladder epithelial cells, for example, the uroplakin II promoter.

- 25 By "recombinant HuAFP" or "rHuAFP" is meant human alpha-fetoprotein encoded by an artificially-constructed nucleic acid.

By "exogenous," as used herein in reference to a gene or a polypeptide, is meant a gene or polypeptide that is not normally present in an

-7-

animal. For example, rHuAFP is exogenous to a goat.

By "purified" is meant that rHuAFP secreted into milk or urine is partially or completely separated from other components (*e.g.*, proteins, lipids, and water) naturally found in milk or urine, thus increasing the effective concentration of rHuAFP relative to unpurified rHuAFP found in milk or urine.

By "substantially pure nucleic acid" is meant nucleic acid that is free of the genes which, in the naturally-occurring genome of the organism from which the nucleic acid of the invention is derived, flank the gene. The term therefore includes, for example, a recombinant DNA that is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote; or which exists as a separate molecule (*e.g.*, a cDNA or a genomic or cDNA fragment produced by PCR or restriction endonuclease digestion) independent of other sequences. It also includes a recombinant DNA that is part of a hybrid gene containing a nucleotide sequence not native to the gene or encoding additional polypeptide sequence, as well as the corresponding mRNA.

By "transformation" or "transfection" or "transduction" is meant any method for introducing foreign molecules into a cell. Lipofection, DEAE-dextran-mediated transfection, microinjection, protoplast fusion, calcium phosphate precipitation, transduction (*e.g.*, bacteriophage, adenoviral retroviral, or other viral delivery), electroporation, and biolistic transformation are just a few of the methods known to those skilled in the art which may be used.

By "transformed cell" or "transfected cell," or "transduced cell," is meant a cell (or a descendent of a cell) into which a DNA molecule encoding rHuAFP has been introduced, by means of recombinant DNA techniques. The DNA molecule may be stably incorporated into the host chromosome, or may be maintained episomally.

By "operably linked" is meant that a gene and one or more

-8-

regulatory sequences are connected in such a way as to permit gene expression when the appropriate molecules (*e.g.*, transcriptional activator proteins) are bound to the regulatory sequences.

By "expression vector" is meant a genetically engineered plasmid or
5 virus, derived from, for example, a bacteriophage, adenovirus, retrovirus, poxvirus, herpesvirus, or artificial chromosome, that is used to transfer an rHuAFP coding sequence, operably linked to a promoter, into a host cell, such that the encoded rHuAFP is expressed within the host cell.

By "embryonal cell" is meant a cell that is capable of being a
10 progenitor to all the somatic and germ-line cells of an organism. Exemplary embryonal cells are embryonic stem cells (ES cells) and fertilized oocytes. Preferably, the embryonal cells of the invention are mammalian embryonal cells.

By "transgene" is meant any piece of nucleic acid that is inserted by
15 artifice into a cell, or an ancestor thereof, and becomes part of the genome of the animal which develops from that cell. Such a transgene may include a gene which is partly or entirely exogenous (*i.e.*, foreign) to the transgenic animal, or may represent a gene having identity to an endogenous gene of the animal.

By "transgenic" is meant any cell that includes a nucleic acid
20 sequence that has been inserted by artifice into a cell, or an ancestor thereof, and becomes part of the genome of the animal which develops from that cell. Preferably, the transgenic animals are transgenic mammals (*e.g.*, goats, sheep, cows, and pigs). Preferably the nucleic acid (transgene) is inserted by artifice into the nuclear genome (*i.e.*, a chromosome), although the transgene may also
25 be episomally maintained (*e.g.*, carried on a vector that contains an origin of replication such as the Epstein-Barr Virus oriP).

By a "leader sequence" or a "signal sequence" is meant a nucleic acid sequence that encodes a protein secretory signal, and, when operably

-9-

linked to a downstream nucleic acid molecule encoding rHuAFP, directs rHuAFP secretion. The leader sequence may be the native rHuAFP leader, an artificially-derived leader, or may be obtained from the same gene as the promoter used to direct transcription of the rHuAFP coding sequence, or from another protein that is normally secreted from a cell.

By "human alpha-fetoprotein secretory signal" or "human alpha-fetoprotein signal peptide" or "human alpha-fetoprotein leader" or "human alpha-fetoprotein signal sequence" is meant a polypeptide having substantially the same amino acid sequence amino acids 1-19 set forth in Genbank Accession No. J00077. The protein secretory signal is cleaved from HuAFP during protein maturation and extracellular secretion.

By "therapeutically-effective amount" is meant an amount of recombinant human alpha-fetoprotein or fragment thereof that when administered to a patient inhibits or stimulates a biological activity modulated by human alpha-fetoprotein. Such biological activities include inhibiting the proliferation of a neoplasm or an autoreactive immune cell, or stimulating proliferation of a cell (*e.g.*, a bone marrow cell). The therapeutically-effective amount may vary depending upon a number of factors, including medical indication, the length of time of administration and the route of administration. For example, rHuAFP can be administered systemically in the range of 0.1 ng - 10g/kg body weight, preferably in the range of 1 ng - 1g/kg body weight, more preferably in the range of 10 ng - 100mg/kg body weight, and most preferably in the range of 1µg-10 mg/kg body weight.

Brief Description of the Drawings

Figure 1 is a diagram showing the structure of a goat beta-casein/rHuAFP transgene for expression and secretion of rHuAFP into milk.

Figure 2 is a diagram showing the genomic organization of the

human AFP gene and the two overlapping lambda (λ) fragments.

Figure 3 is a diagram showing the design of a vector including the 5' subclone of the human AFP gene and an expression construct.

Figure 4 is a diagram showing the design of a vector including the 3' subclone of the human AFP gene and an expression construct.

Figure 5 is a diagram showing a strategy for linking the 5' and 3' AFP gene fragments and inserting the entire human AFP genomic fragment into the GTC beta-casein expression vector.

Detailed Description of the Invention

The present invention features a process for expressing secreted recombinant human alpha-fetoprotein (rHuAFP) in transgenic mammals, particularly ruminants (*e.g.*, cattle, sheep, and goats). The transgene that directs expression of secreted rHuAFP contains the human AFP coding region fused downstream of a nucleic acid containing a transcriptional promoter. Between the promoter and the protein coding region is a leader sequence encoding a protein secretory signal. Depending upon the promoter and secretory signal employed, the transgene-encoded rHuAFP is secreted into the milk or urine of the transgenic animal. Additional nucleic acid elements, such as transcriptional enhancers, transcriptional and translational terminator sequences, 3' untranslated regions that enhance mRNA stability, and introns that enhance expression may also be included in the transgenic construct.

Production of rHuAFP by secretion into milk or urine facilitates its purification and obviates removal of blood products and culture medium additives, some of which may be toxic, carcinogenic, or infectious. Moreover, milk containing rHuAFP may be directly consumed by humans or other mammals. Expression of rHuAFP in urine allows the use of both male and female animals for rHuAFP production. In addition, rHuAFP is produced as

soon as the animals begin to produce urine. Finally, purification of rHuAFP from urine is relatively straightforward, as urine normally contains a low protein content.

Transgene constructs

5 Useful promoters for the expression of a rHuAFP transgene in mammary tissue include promoters that naturally drive the expression of mammary-specific proteins, such as milk proteins, although any promoter that permits secretion of the transgene product into milk may be used. These include, *e.g.*, the promoters that naturally direct expression of whey acidic
10 protein (WAP), alpha S1-casein, alpha S2-casein, beta-casein, kappa-casein, beta-lactoglobulin, and alpha-lactalbumin (see, *e.g.*, Drohan *et al.*, U.S.P.N. 5,589,604; Meade *et al.* U.S. Patent No. 4, 873,316; and Karatzas *et al.*, U.S. patent No. 5,780,009).

15 A useful promoter for the expression of an rHuAFP transgene in urinary tissue is the uroplakin promoter (Kerr *et al.*, *Nat. Biotechnol.* 16:75-79, 1998), although any promoter that permits secretion of the transgene product into urine may be used.

20 The transgene construct preferably includes a leader sequence downstream from the promoter. The leader sequence is a nucleic acid sequence that encodes a protein secretory signal, and, when operably linked to a downstream nucleic acid molecule encoding rHuAFP, directs rHuAFP secretion. The leader sequence may be obtained from the same gene as the promoter used to direct transcription of the nucleic acid molecule encoding rHuAFP (for example, a gene that encodes a milk-specific protein).
25 Alternatively, a leader sequence encoding the native rHuAFP protein secretory signal (amino acids 1-19 of Genbank Accession No. J00077) may be employed: nucleotides 45-101 of Genbank Accession No. J00077 encode the native

HuAFP protein secretory signal. Other options include use of a leader sequence that encodes a protein secretory signal from any other protein that is normally secreted from a cell, an artificial leader sequence that encodes an artificial protein secretory signal, or a hybrid leader sequence (*e.g.*, a fusion of the goat beta-casein and HuAFP leader sequences).

In addition, the transgene construct preferably includes a transcription termination site, a signal for polyadenylation of the transcribed mRNA, and a translation termination signal. The transgene may also encode any 3' untranslated region (UTR), which increases stability of the rHuAFP mRNA, for example, a 3' UTR from the bovine growth hormone gene, a milk protein gene, or a globin gene.

The transgene construct may also include a transcriptional enhancer upstream or downstream from the transcribed region of the transgene, such as an enhancer from a viral (*e.g.*, SV40) or mammalian (*e.g.*, casein) gene.

The transgene construct may further include an intron that increases the level of expression of the transgene. The intron may be placed between the transcription initiation site and the translational start codon, 3' of the translational stop codon, or within the coding region of the transgene. The intron should include a 5' splice site (*i.e.*, a donor site), a 3' splice site (*i.e.*, an acceptor site), and preferably, at least 100 nucleotides between the two sites. Any intron that is known in the art to increase expression of a transgene (*e.g.*, an intron from a ruminant casein gene) may be used.

The transgene construct may include genomic or cDNA that expresses HuAFP or a fragment thereof. Exemplary fragments of HuAFP are described in Murgita, WO 96/2287. In addition, the transgene may be engineered to express a rHuAFP molecule that is non-glycosylated. This is accomplished by mutating the codon encoding the single N-linked glycosylation site of the AFP molecule using standard methods known in the

art.

The rHuAFP transgene may be carried within a circular plasmid, a cosmid vector, or other vector, such as a vector derived from a virus. The vector may contain additional sequences that facilitate its propagation in prokaryotic and eukaryotic cells, for example, drug-selectable markers (*e.g.*, for ampicillin resistance in *E. coli*, or G-418 resistance in mammalian cells) and origins of replication (*e.g.*, colE1 for replication in prokaryotic cells, and oriP for replication in mammalian cells).

Generation of Transgenic Animals

Transgenic constructs are usually introduced into cells by microinjection (Ogata *et al.*, U.S. Patent No. 4,873,292). A microinjected embryo is then transferred to an appropriate female resulting in the birth of a transgenic or chimeric animal, depending upon the stage of development of the embryo when the transgene integrated. Chimeric animals can be bred to form true germline transgenic animals.

In some methods of transgenesis, transgenes are introduced into the pronuclei of fertilized oocytes. For some animals, such as mice, fertilization is performed *in vivo* and fertilized ova are surgically removed. In other animals, the ova can be removed from live, or from newly-dead (*e.g.*, slaughterhouse) animals and fertilized *in vitro*.

Alternatively, transgenes can be introduced into embryonic stem cells (ES cells). Transgenes can be introduced into such cells by electroporation, microinjection, or any other techniques used for the transfection of cells which are known to the skilled artisan. Transformed cells are combined with blastocysts from the animal from which they originate. The transformed cells colonize the embryo, and in some embryos these cells form the germline of the resulting chimeric animal (Jaenisch, R., *Science* 240:

1468-1474, 1988).

ES cells containing an rHuAFP transgene may also be used as a source of nuclei for transplantation into an enucleated fertilized oocyte, thus giving rise to a transgenic animal. More generally, any diploid cell derived from embryonic, fetal, or adult tissue and containing an rHuAFP transgene may be introduced into an enucleated unfertilized egg. The cloned embryo is implanted and gestated within an appropriate female, thus resulting in a fully transgenic animal (Wilmut *et al.*, *Nature* 385:810-813, 1997).

In general, expression of any transgene depends upon its integration position and copy number. After a transgenic animal having the appropriate transgene expression level and tissue-specific transgene expression pattern is obtained by traditional methods (*e.g.*, pronuclear injection or generation of chimeric embryos), the animal is bred in order to obtain progeny having the same transgene expression level and pattern. There are several limitations to this approach. First, transmission of the transgene to offspring does not occur in transgenic chimeras lacking transgenic germ cells. Second, because a heterozygous transgenic founder is bred with a non-transgenic animal, only half of the progeny will be transgenic. Third, the number of transgenic progeny is further limited by the length of the gestation period and number of offspring per pregnancy. Finally, the number of useful transgenic progeny may be further limited by gender: for example, only female animals are useful for producing rHuAFP expressed in milk. In view of these limitations, nuclear transfer technology provides the advantage of allowing, within a relatively short time period, the generation of many female transgenic animals that are genetically identical.

Animals expressing rHuAFP in their milk also may be generated by direct transfer of the transgene into the mammary tissue of post-partum animals (Karatzas *et al.*, U.S. patent No. 5,780,009). Such animals do not contain the

transgene within their germline, and hence do not give rise to transgenic progeny.

Screening for Transgenic Animals

After the candidate transgenic animals are generated, they must be
5 screened in order to detect animals whose cells contain and express the
transgene. The presence of a transgene in animal tissues is typically detected
by Southern blot analysis or by employing PCR-amplification of DNA from
candidate transgenic animals (see, *e.g.*, Ausubel *et al.*, *Current Protocols in*
Molecular Biology, John Wiley & Sons, New York, NY, 1998; see also Lubon
10 *et al.*, U.S.P.N. 5,831,141). rHuAFP expression in milk or urine may be
determined by any standard immunological assay, for example, ELISA or
Western blotting analysis, using an anti-AFP antibody (see, *e.g.*, Murgita *et al.*,
U.S.P.N. 5,384,250 and Ausubel *et al.*, *supra*). For a working example of
ELISA-based detection of transgene-encoded protein in milk, see Drohan *et al.*,
15 U.S.P.N. 5,589,604.

Purification of AFP from Urine or Milk

Recombinant protein may be purified from milk or urine using
standard protein purification techniques, such as affinity chromatography (see,
e.g., Ausubel *et al.*, *Current Protocols in Molecular Biology*, John Wiley &
20 Sons, New York, NY, 1998; see also Lubon *et al.*, U.S.P.N. 5,831,141) or other
methods known to those skilled in the art of protein purification. Once isolated,
the recombinant protein can, if desired, be further purified by *e.g.*, by high
performance liquid chromatography (HPLC; *e.g.*, see Fisher, *Laboratory*
Techniques In Biochemistry And Molecular Biology, eds. Work and Burdon,
25 Elsevier, 1980). Preferably, the purification is by at least 2-fold, more
preferably, by at least 10-fold, still more preferably, by at least 100-fold, and

most preferably, by at least 1000-fold.

Use of rHuAFP Purified from Milk or Urine of Transgenic Animals

rHuAFP (Murgita *et al.*, U.S.P.N. 5,384,250) in milk or urine or purified from milk or urine may be used as a diagnostic standard (*e.g.*, for
5 detection of increased levels of AFP in adult human serum, which may indicate the presence of cancer or liver regeneration) or as a therapeutic. For example, rHuAFP produced by the methods of the invention may be administered to mammals to inhibit cancer cell growth, to induce bone marrow cell
proliferation (for example, after a bone marrow transplant or after
10 administration of a myelotoxic treatment such as chemotherapy), or as an immunosuppressive agent (for example, to treat systemic lupus erythematosus, myasthenia gravis, insulin-dependent diabetes mellitus, or to inhibit rejection of a transplanted organ).

rHuAFP in milk or urine or purified from milk or urine may be
15 administered in an effective amount either alone or in combination with a pharmaceutically acceptable carrier or diluent, either alone or in combination with other therapeutic agents by any convenient means known to skilled artisans, *e.g.*, intravenously, orally, intramuscularly, or intranasally.

20 Example I: Generation of Transgenic Goats Expressing Recombinant Human AFP (rHuAFP)

Transgene construction and generation of transgenic goats

Transgenic goats expressing rHuAFP in their milk, under the control
of the goat beta-casein promoter, are generated as follows. A DNA fragment
25 containing the full length coding region of human AFP and lacking the translational start sequence is obtained by performing polymerase chain reaction (PCR) amplification using a plasmid containing the HuAFP cDNA

(Genbank Accession No. J00077), such as pHuAFP (described in Murgita *et al.*, U.S.P.N. 5,384,250) as a template and the following oligonucleotide primers: NH₂ (5'-AAA CTC GAG AAG TGG GTG GAA-3') and COOH (5'-AAA CTC GAG TTA AAC TCC CAA AGC-3').

- 5 Each PCR reaction contains 34 µl DNA template, 10 µl of 10 pmol/µl 5'-primer, 10 µl 10X reaction buffer, 20 µl 1mM dNTP's, 2 µl DMSO and 1 µl DNA template, 10 µl of 10 pmol/µl of 10 pmol/µl 5'-primer, 10 µl of 10 pmol/µl 3'-primer, 1 µl glycerol, 10 µl DMSO and 1 µl *Pfu* DNA polymerase. Annealing, extension, and denaturation temperatures are 50°C, 10 72°C and 94°C, respectively, for 30 cycles, using the Gene Amp PCR System 9600. The 1783-bp DNA obtained from the PCR reactions is digested with *Xho* I and then purified by isolating the fragment from a 0.7% TAE agarose gel, followed by gel extraction employing the Geneclean method (Bio 10l; Vista, CA) according to the manufacturer's instructions.

- 15 The transgene vector (see Figure 1; see Meade *et al.*, U.S.P.N. 5,827,690) contains an altered goat beta-casein gene with an *Xho* I site in place of the coding portion of the gene. The portion deleted from the goat beta-casein gene extends from the *Taq* I site in exon 2 to the *Ppu* MI site in exon 7. Exon 2 contains the translational start codon in addition to a 15 amino acid 20 secretion signal. To generate the goat beta-casein/human AFP transgene, the *Xho* I/*Xho* I HuAFP cDNA is ligated between exons 2 and 7 of the goat beta-casein gene at the *Xho* I site. The complete transgene contains 6.2 kb of 5' goat beta-casein sequence, the 1.8 kb HuAFP cDNA, and the 7.1 kb 3' goat beta-casein flanking sequence.

- 25 Transgenic goats are generated by injecting, into the pronucleus of collected embryos, the 15.1 kb fragment of the goat beta-casein-HuAFP purified free from procaryotic DNA at a concentration of 1.0 µg/ml in 10 mM Tris, pH 7.5, 0.1 mM EDTA. Injected embryos are then transferred to recipient

-18-

- females. A founder (F_0) transgenic goat is identified by analyzing genomic DNA from blood by polymerase chain reaction (PCR) and by Southern blot analysis in order to detect the presence of the transgene. For PCR analysis, the same two oligonucleotides that are employed to generate the HuAFP cDNA are
- 5 used in the reaction. For Southern blot analysis, the DNA is fractionated on a 1% TBE agarose gel, blotted onto nitrocellulose, and probed with a random-primed ^{32}P -labelled 1.8 kb HuAFP cDNA. The identified founder is then bred to a nontransgenic animal to produce transgenic offspring. Alternatively, transgenic offspring may be obtained by nuclear transfer, as described above.
- 10 Transmission of the transgene is detected by analyzing genomic DNA from blood as described above.

Lactation induction

- Female animals twelve months of age or older are induced to lactate by hormone therapy and hand stimulation over a 12 day period. During the
- 15 first 4 days, the animal receive subcutaneous injections of 0.1 mg/kg of estradiol 17- β and 0.25 mg/kg of progesterone dissolved in 100% ethanol. This daily amount is divided between morning and evening injections. The udder is palpated once daily and the teats are hand-stimulated for 5-10 minutes each morning. Lactating transgenic females are milked manually twice per day and
- 20 the milk is stored frozen at -20°C .

Protein purification

- Transgenic goat milk containing rHuAFP is thawed and the pH adjusted to 4.4 with glacial acetic acid to precipitate out the casein. The resultant precipitate is removed by centrifugation at $8000 \times g$ for 20 min. at
- 25 4°C . The supernant is adjusted to pH 5.5 with NaOH and filtered through a $22 \mu\text{m}$ filter. The rHuAFP is purified from the whey fraction by applying the

-19-

filtrate to a Butyl-Toyopearl column which is equilibrated in 0.2 M sodium phosphate, 0.1 M arginine-HCl, 0.01% Tween 80, pH 6.0. The rHuAFP is eluted with a solution of 0.2 M sodium phosphate, 0.1 M arginine-HCl, 70% ethylene glycol. Fractions containing rHuAFP, determined by Western blot or

5 ELISA, are pooled and dialyzed against 30 mM Tris-HCl, pH 8.0. Final purification of rHuAFP is achieved by applying the dialyzed sample onto a Mono Q column equilibrated in 20 mM Tris-HCl, pH 8.0. Bound proteins are eluted during a step gradient from 0-100% 91 M NaCl, 20 mM Tris-HCl, pH 8.0).

10 Example II: Design of a Genomic Alpha Fetoprotein Transgene Expression Construct

Human AFP Gene Cloning

The gene for human AFP spans roughly 19 kb and contains 15 exons (14 coding) separated by 14 introns. The complete sequence of the human AFP

15 gene has been reported by Gibbs *et al.* (*Biochemistry* 26:1332-1343, 1987) and set forth in GenBank Accession No. M16610. The gene was initially cloned in two fragments of approximately 15 kb, which were then combined, to generate the expressed protein.

A human placental genomic library (Stratagene, La Jolla, CA), with

20 an average insert size of between 9 and 23 kb, was initially screened with a series of complementary oligonucleotide probes which recognize exons at the beginning, middle, and end of the human AFP gene. The first screen did not produce any positive clones. Two larger DNA probes were then made by using the polymerase chain reaction (PCR) to amplify regions of the beginning and

25 end of the AFP gene from human genomic DNA (arrows, Figure 2). Subsequent screening of the library with these probes produced two overlapping lambda (λ) phage clones, of approximately 15 kb, which together

-20-

span the length of the human AFP gene (Figure 2).

Construct Design

The two phage inserts were then subcloned into a superCOS 1 vector (this vector was used because it can accommodate larger DNA inserts). The two resulting subclones, gtc912 and gtc913 are then manipulated, as follows, to generate the final expression constructs. First, sequences 5' and 3' of the coding region are removed. In addition, at the 5' end, a Kozak sequence is added to ensure efficient initiation of translation. This is accomplished by inserting restriction enzyme "linkers" into the gene sequences for the subsequent excision of the appropriate sequences, leaving the flanking sequences intact (Figures 3&4). Second, the 5' and 3' pieces are excised from their respective vectors using an enzyme common to the two inserts which allows them to be joined together to form the complete gene. The enzyme BglI, is used since it cuts once at the 3' end of the 5' piece (IK179) and once, at the same site, at the 5' end of the 3' piece (IK175). Finally, these two pieces are linked together in a superCos plasmid vector in the SalI site and then the entire genomic fragment is placed into the SalI site of a GTC beta-casein expression vector (Figure 5).

The genomic AFP gene construct, if desired, may be mutated at its single N-linked glycosylation site. Using restriction sites flanking the glycosylation site (*e.g.*, DsaI and BlnI), an oligonucleotide containing the mutation (N to Q) can be substituted using standard molecular biological techniques (*e.g.*, gapped mutagenesis). The non-glycosylated version of the genomic AFP is then ligated into the beta-casein vector as described above and used to generate a transgenic animal, *e.g.*, a mouse, goat, sheep, pig, or cow.

The publications listed hereafter describe the generation, detection, and analysis of transgenic animals that secrete recombinant proteins into milk, as well as purification of the recombinant proteins. These publications are

-21-

herein incorporated by reference: Hurwitz *et al.*, U.S.P.N. 5,648,243 (goats); Meade, *et al.*, U.S.P.N. 5,827,690 (goats); DiTullio *et al.*, U.S.P.N. 5,843,705 (goats); Clark *et al.*, U.S.P.N. 5,322,775 (sheep); Garner *et al.*, U.S.P.N. 5,639,940 (sheep); Deboer *et al.*, U.S.P.N. 5,633,076 (cows); and Drohan *et al.*,
5 U.S.P.N. 5,589,604 (pigs and mice). Kerr *et al.*, *Nat. Biotechnol.* 16:75-79, 1998, herein incorporated by reference, describes the generation and analysis of transgenic animals that excrete recombinant proteins into urine, as well as purification of the recombinant proteins.

All publications and patent applications mentioned in this
10 specification are herein incorporated by reference to the same extent as if each independent publication or patent application was specifically and individually indicated to be incorporated by reference.

While the invention has been described in connection with specific
embodiments thereof, it will be understood that it is capable of further
15 modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure that come within known or customary practice within the art to which the invention pertains and may be applied to the essential features hereinbefore set forth.

20 What is claimed is:

-22-

CLAIMS

1. A substantially pure nucleic acid molecule comprising: (i) a nucleic acid sequence encoding recombinant human alpha-fetoprotein (rHuAFP), (ii) a milk-specific promoter, said promoter being operably linked to
5 said rHuAFP-encoding sequence, and (iii) a leader sequence encoding a protein secretory signal that enables secretion of said rHuAFP by milk-producing cells into the milk of a mammal.
2. A substantially pure nucleic acid molecule comprising: (i) a nucleic acid sequence encoding recombinant human alpha-fetoprotein
10 (rHuAFP), (ii) a urine-specific promoter, said promoter being operably linked to said rHuAFP-encoding sequence, and (iii) a leader sequence encoding a protein secretory signal that enables secretion of said rHuAFP by urine-producing cells into the urine of a mammal.
3. A non-human transgenic mammal that expresses recombinant
15 human alpha-fetoprotein (rHuAFP) in its milk, wherein milk-producing cells of said mammal contain a transgene that comprises: (i) a nucleic acid sequence encoding rHuAFP, (ii) a milk-specific promoter, said promoter being operably linked to said rHuAFP-encoding sequence, and (iii) a leader sequence encoding a protein secretory signal that enables secretion of said rHuAFP by milk-
20 producing cells into the milk of a mammal.
4. A non-human transgenic mammal that expresses recombinant human alpha-fetoprotein (rHuAFP) in its urine, wherein urine-producing cells of said mammal contain a transgene that comprises: (i) a nucleic acid sequence encoding rHuAFP, (ii) a urine-specific promoter, said promoter being operably

-23-

linked to said rHuAFP-encoding sequence, and (iii) a leader sequence encoding a protein secretory signal that enables secretion of said rHuAFP by urine-producing cells into the urine of an animal.

5 5. The non-human transgenic mammal of claim 1 or 2, wherein the mammal is a goat, a cow, a sheep, or a pig.

6. Non-human mammal's milk comprising recombinant human alpha-fetoprotein (rHuAFP).

10 7. The milk of claim 6, wherein the rHuAFP is soluble and is produced by a non-human transgenic mammal whose milk-producing cells express a transgene that comprises: (i) a nucleic acid sequence encoding rHuAFP, (ii) a milk-specific promoter, said promoter being operably linked to said rHuAFP-encoding sequence, and (iii) a leader sequence encoding a protein secretory signal that enables secretion of said rHuAFP by said milk-producing cells into the milk of said mammal.

15 8. Non-human mammal's urine comprising recombinant human alpha-fetoprotein (rHuAFP).

20 9. The urine of claim 8, wherein the rHuAFP is soluble and is produced by a non-human transgenic mammal whose urine-producing cells express a transgene that comprises: (i) a nucleic acid sequence encoding rHuAFP, (ii) a urine-specific promoter, said promoter being operably linked to said rHuAFP-encoding sequence, and (iii) a leader sequence encoding a protein secretory signal that enables secretion of said rHuAFP by said urine-producing cells into the urine of said mammal.

10. A method of producing recombinant human alpha-fetoprotein (rHuAFP) that is secreted in the milk of a mammal, said method comprising the steps of:

- 5 (a) providing a cell transfected with a transgene that comprises: (i) a nucleic acid sequence encoding rHuAFP, (ii) a milk-specific promoter, said promoter being operably linked to said rHuAFP-encoding sequence, and (iii) a leader sequence encoding a protein secretory signal that enables secretion of said rHuAFP by a milk-producing cell, wherein said milk-producing cell is derived from said transfected cell;
- 10 (b) growing said cell to produce a mammal comprising milk-producing cells that express and secrete said rHuAFP into said milk; and
- (c) collecting said milk containing said rHuAFP from said mammal.

11. The method of claim 10, wherein said rHuAFP is purified from said milk.

15 12. A method of producing recombinant human alpha-fetoprotein (rHuAFP) that is secreted in the urine of a mammal, said method comprising the steps of:

- 20 (a) providing a cell transfected with a transgene that comprises: (i) a nucleic acid sequence encoding rHuAFP, (ii) a urine-specific promoter, said promoter being operably linked to said rHuAFP-encoding sequence, and (iii) a leader sequence encoding a protein secretory signal that enables secretion of said rHuAFP by a urine-producing cell, wherein said urine-producing cell is derived from said transfected cell;
- 25 (b) growing said cell to produce a mammal comprising urine-producing cells that express and secrete said rHuAFP into said urine; and
- (c) collecting said urine containing said rHuAFP from said mammal.

13. The method of claim 12, wherein said rHuAFP is purified from said urine.

14. A method of treating a patient in need of recombinant human alpha-fetoprotein (rHuAFP), said method comprising administering to said
5 patient a therapeutically-effective amount of non-human mammal's milk comprising recombinant human alpha-fetoprotein (rHuAFP).

15 10 15 16. The method of claim 14, wherein said rHuAFP is produced by a non-human transgenic mammal whose milk-producing cells contain a transgene that comprises: (i) a nucleic acid sequence encoding rHuAFP, (ii) a milk-specific promoter, said promoter being operably linked to said rHuAFP-encoding sequence, and (iii) a leader sequence encoding a protein secretory signal that enables secretion of said rHuAFP by milk-producing cells into the milk of said mammal.

17 16. A method of treating a patient in need of recombinant human alpha-fetoprotein (rHuAFP), said method comprising administering to said
15 patient a therapeutically-effective amount of recombinant human alpha-fetoprotein (rHuAFP) purified from a non-human mammal's urine.

18 17. The method of claim 16, wherein said rHuAFP is produced by a non-human transgenic mammal whose urine-producing cells contain a
20 transgene that comprises: (i) a nucleic acid sequence encoding rHuAFP, (ii) a urine-specific promoter, said promoter being operably linked to said rHuAFP-encoding sequence, and (iii) a leader sequence encoding a protein secretory signal that enables secretion of said rHuAFP by urine-producing cells into the urine of said mammal.

-26-

18. The method of claim 14 or 16, wherein said method is for the treatment of cancer.

19. The method of claim 14 or 16, wherein said method is for suppressing the immune system of a patient in need thereof.

5 20. The method of claim 14 or 16, wherein said method is for inducing proliferation of bone marrow cells in a patient in need thereof.

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification : C12N		A2	(11) International Publication Number: WO 00/40693
			(43) International Publication Date: 13 July 2000 (13.07.00)
(21) International Application Number: PCT/US00/00264			(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
(22) International Filing Date: 6 January 2000 (06.01.00)			
(30) Priority Data: 60/114,995 6 January 1999 (06.01.99) US			
(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application US 60/114,995 (CIP) Filed on 6 January 1999 (06.01.99)			
(71) Applicant (for all designated States except US): ATLANTIC BIOPHARMACEUTICALS, INC. [US/US]; 50 Church Street, Cambridge, MA 02138 (US).			
(72) Inventors; and			Published <i>Without international search report and to be republished upon receipt of that report.</i>
(75) Inventors/Applicants (for US only): LINDSAY, Stace [US/US]; 8 Cypress Street, Cambridge, MA 02140 (US). MULROY, Robert [US/US]; 19 Hilliard Street, Cambridge, MA 02138 (US). SEMENTIUK, Daniel [US/US]; 1860 Thousand Oaks Lane, Lawrenceville, GA 30043 (US).			
(74) Agent: CLARK, Paul, T.; Clark & Elbing LLP, 176 Federal Street, Boston, MA 02110-2214 (US).			
(54) Title: EXPRESSION OF SECRETED HUMAN ALPHA-FETOPROTEIN IN TRANSGENIC ANIMALS			
(57) Abstract The invention features a process of expressing secreted recombinant human alpha-fetoprotein (rHuAFP) in the milk or urine of transgenic mammals.			

1/4

— GOAT BETA CASEIN
 ▨ HUMAN AFP CDNA

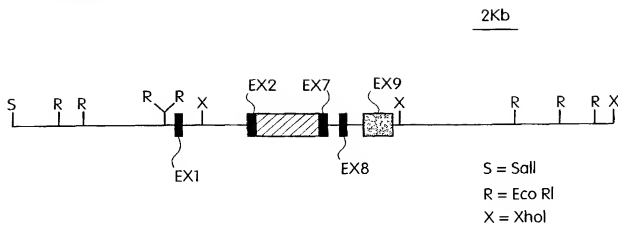


Fig. 1

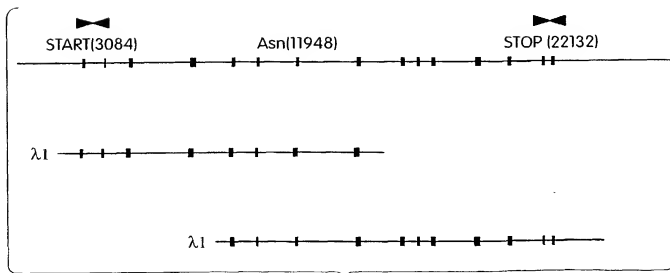


Fig. 2

2/4

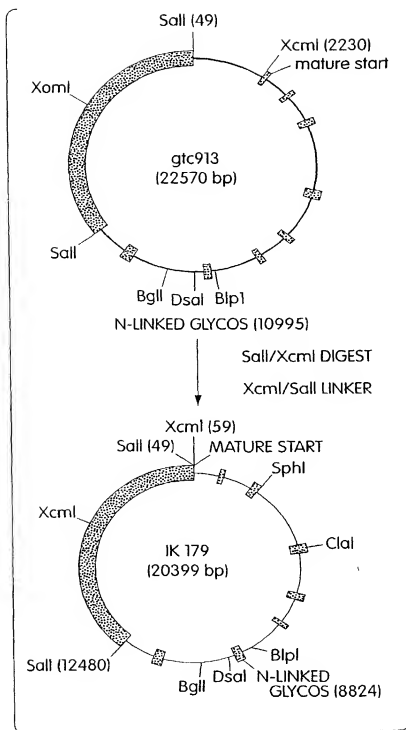


Fig. 3

3/4

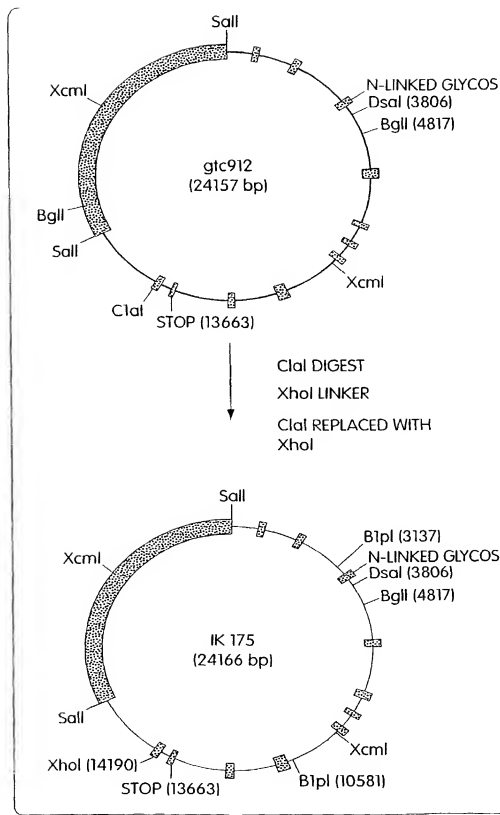


Fig. 4

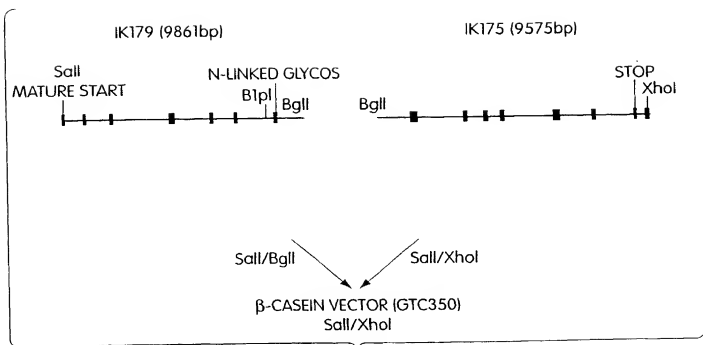


Fig. 5

PATENT
ATTORNEY DOCKET NO: 06727/008002

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled **EXPRESSION OF SECRETED HUMAN ALPHA-FETOPROTEIN IN TRANSGENIC ANIMALS**, the specification of which

☒ is attached hereto.

☐ was filed on _____ as Application Serial No. _____
 and was amended on _____

☐ was described and claimed in PCT International Application No. _____
 filed on _____ and as amended under PCT Article 19 on _____

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information I know to be material to patentability in accordance with Title 37, Code of Federal Regulations, § 1.56.

FOREIGN PRIORITY RIGHTS: I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

Country	Serial Number	Filing Date	Priority Claimed?
PCT	PCT/US00/00264	06 January 2000	Yes

PROVISIONAL PRIORITY RIGHTS: I hereby claim priority benefits under Title 35, United States Code, § 119(e) and § 120 of any United States provisional patent application(s) listed below filed by an inventor or inventors on the same subject matter as the present application and having a filing date before that of the application(s) of which priority is claimed:

Serial Number	Filing Date	Status
60/114,995	06 January 1999	Abandoned

COMBINED DECLARATION AND POWER OF ATTORNEY

NON-PROVISIONAL PRIORITY RIGHTS: I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose all information I know to be material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

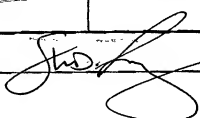
Serial Number	Filing Date	Status

I hereby appoint the following attorneys and/or agents to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: Paul T. Clark, Reg. No. 30,162, Karen L. Elbing, Ph.D. Reg. No. 35,238, Kristina Bieker-Brady, Ph.D. Reg. No. 39,109, Susan M. Michaud, Ph.D. Reg. No. 42,885, James D. DeCamp, Ph.D., Reg. No. 43,580, Sean J. Edman, Reg. No. 42,506, Timothy J. Douros, Reg. No. 41,716.

Address all telephone calls to: Paul T. Clark at 617/428-0200.

Address all correspondence to: Paul T. Clark at Clark & Elbing LLP, 176 Federal Street, Boston, MA 02110. Customer No: 21559

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Full Name (First, Middle, Last)	Residence Address (City, State, Country)	Post Office Address (Street, City, State, Country)	Citizenship
Stace Lindsay	Cambridge, MA U.S.A.	8 Cypress Street Cambridge, MA 02140 U.S.A.	U.S.A.
Signature: 			Date: 10/11/01

COMBINED DECLARATION AND POWER OF ATTORNEY

2-00

Full Name (First, Middle, Last)	Residence Address (City, State, Country)	Post Office Address (Street, City, State, Country)	Citizenship
<u>Robert Mulroy</u>	Cambridge, MA U.S.A.	19 Hilliard Street Cambridge, MA 02138 U.S.A. <i>MA</i>	U.S.A.
Signature: <i>Robert Mulroy</i>			Date: 7/4/01

3-00

Full Name (First, Middle, Last)	Residence Address (City, State, Country)	Post Office Address (Street, City, State, Country)	Citizenship
<u>Daniel Semenluk</u>	Lawrenceville, GA	1860 Thousand Oaks Lane Lawrenceville, GA 30043 U.S.A. <i>GA</i>	U.S.A.
Signature: <i>D. Semenluk</i>			Date: 12/10/2001

SEQUENCE LISTING

<110> Lindsay, Stace
Mulroy, Robert
Semeniuk, Daniel

<120> Expression of Human Alpha-Fetoprotein in
Mammalian Cells

<130> 06727/008002

<150> PCT/US00/00264

<151> 2000-01-06

<150> US 60/114,995

<151> 1999-01-06

<160> 7

<170> FastSEQ for Windows Version 4.0

<210> 1

<211> 609

<212> PRT

<213> Homo sapiens

<400> 1

```
Met Lys Trp Val Glu Ser Ile Phe Leu Ile Phe Leu Leu Asn Phe Thr
1      5      10      15
Glu Ser Arg Thr Leu His Arg Asn Glu Tyr Gly Ile Ala Ser Ile Leu
20     25     30
Asp Ser Tyr Gln Cys Thr Ala Glu Ile Ser Leu Ala Asp Leu Ala Thr
35     40     45
Ile Phe Phe Ala Gln Phe Val Gln Glu Ala Thr Tyr Lys Glu Val Ser
50     55     60
Lys Met Val Lys Asp Ala Leu Thr Ala Ile Glu Lys Pro Thr Gly Asp
65     70     75     80
Glu Gln Ser Ser Gly Cys Leu Glu Asn Gln Leu Pro Ala Phe Leu Glu
85     90     95
Glu Leu Cys His Glu Lys Glu Ile Leu Glu Lys Tyr Gly His Ser Asp
100    105    110
Cys Cys Ser Gln Ser Glu Glu Gly Arg His Asn Cys Phe Leu Ala His
115    120    125
Lys Lys Pro Thr Pro Ala Ser Ile Pro Leu Phe Gln Val Pro Glu Pro
130    135    140
Val Thr Ser Cys Glu Ala Tyr Glu Glu Asp Arg Glu Thr Phe Met Asn
145    150    155    160
Lys Phe Ile Tyr Glu Ile Ala Arg Arg His Pro Phe Leu Tyr Ala Pro
165    170    175
Thr Ile Leu Leu Trp Ala Ala Arg Tyr Asp Lys Ile Ile Pro Ser Cys
180    185    190
Cys Lys Ala Glu Asn Ala Val Glu Cys Phe Gln Thr Lys Ala Ala Thr
195    200    205
Val Thr Lys Glu Leu Arg Glu Ser Ser Leu Leu Asn Gln His Ala Cys
210    215    220
Ala Val Met Lys Asn Phe Gly Thr Arg Thr Phe Gln Ala Ile Thr Val
225    230    235    240
Thr Lys Leu Ser Gln Lys Phe Thr Lys Val Asn Phe Thr Glu Ile Gln
```

245 250 255
 Lys Leu Val Leu Asp Val Ala His Val His Glu His Cys Cys Arg Gly
 260 265 270
 Asp Val Leu Asp Cys Leu Gln Asp Gly Glu Lys Ile Met Ser Tyr Ile
 275 280 285
 Cys Ser Gln Gln Asp Thr Leu Ser Asn Lys Ile Thr Glu Cys Cys Lys
 290 295 300
 Leu Thr Thr Leu Glu Arg Gly Gln Cys Ile Ile His Ala Glu Asn Asp
 305 310 315 320
 Glu Lys Pro Glu Gly Leu Ser Pro Asn Leu Asn Arg Phe Leu Gly Asp
 325 330 335
 Arg Asp Phe Asn Gln Phe Ser Ser Gly Glu Lys Asn Ile Phe Leu Ala
 340 345 350
 Ser Phe Val His Glu Tyr Ser Arg Arg His Pro Gln Leu Ala Val Ser
 355 360 365
 Val Ile Leu Arg Val Ala Lys Gly Tyr Gln Glu Leu Leu Glu Lys Cys
 370 375 380
 Phe Gln Thr Glu Asn Pro Leu Glu Cys Gln Asp Lys Gly Glu Glu Glu
 385 390 395 400
 Leu Gln Lys Tyr Ile Gln Glu Ser Gln Ala Leu Ala Lys Arg Ser Cys
 405 410 415
 Gly Leu Phe Gln Lys Leu Gly Glu Tyr Tyr Leu Gln Asn Ala Phe Leu
 420 425 430
 Val Ala Tyr Thr Lys Lys Ala Pro Gln Leu Thr Ser Ser Glu Leu Met
 435 440 445
 Ala Ile Thr Arg Lys Met Ala Ala Thr Ala Ala Thr Cys Cys Gln Leu
 450 455 460
 Ser Glu Asp Lys Leu Leu Ala Cys Gly Glu Gly Ala Ala Asp Ile Ile
 465 470 475 480
 Ile Gly His Leu Cys Ile Arg His Glu Met Thr Pro Val Asn Pro Gly
 485 490 495
 Val Gly Gln Cys Cys Thr Ser Ser Tyr Ala Asn Arg Arg Pro Cys Phe
 500 505 510
 Ser Ser Leu Val Val Asp Glu Thr Tyr Val Pro Pro Ala Phe Ser Asp
 515 520 525
 Asp Lys Phe Ile Phe His Lys Asp Leu Cys Gln Ala Gln Gly Val Ala
 530 535 540
 Leu Gln Thr Met Lys Gln Glu Phe Leu Ile Asn Leu Val Lys Gln Lys
 545 550 555 560
 Pro Gln Ile Thr Glu Glu Gln Leu Glu Ala Val Ile Ala Asp Phe Ser
 565 570 575
 Gly Leu Leu Glu Lys Cys Cys Gln Gly Gln Glu Gln Glu Val Cys Phe
 580 585 590
 Ala Glu Glu Gly Gln Lys Leu Ile Ser Lys Thr Arg Ala Ala Leu Gly
 595 600 605
 Val

<210> 2
 <211> 590
 <212> PRT
 <213> Homo sapiens

<400> 2
 Thr Leu His Arg Asn Glu Tyr Gly Ile Ala Ser Ile Leu Asp Ser Tyr
 1 5 10 15
 Gln Cys Thr Ala Glu Ile Ser Leu Ala Asp Leu Ala Thr Ile Phe Phe
 20 25 30

Ala Gln Phe Val Gln Glu Ala Thr Tyr Lys Glu Val Ser Lys Met Val
35 40 45
Lys Asp Ala Leu Thr Ala Ile Glu Lys Pro Thr Gly Asp Glu Gln Ser
50 55 60
Ser Gly Cys Leu Glu Asn Gln Leu Pro Ala Phe Leu Glu Glu Leu Cys
65 70 75 80
His Glu Lys Glu Ile Leu Glu Lys Tyr Gly His Ser Asp Cys Cys Ser
85 90 95
Gln Ser Glu Glu Gly Arg His Asn Cys Phe Leu Ala His Lys Lys Pro
100 105 110
Thr Pro Ala Ser Ile Pro Leu Phe Gln Val Pro Glu Pro Val Thr Ser
115 120 125
Cys Glu Ala Tyr Glu Glu Asp Arg Glu Thr Phe Met Asn Lys Phe Ile
130 135 140
Tyr Glu Ile Ala Arg Arg His Pro Phe Leu Tyr Ala Pro Thr Ile Leu
145 150 155 160
Leu Trp Ala Ala Arg Tyr Asp Lys Ile Ile Pro Ser Cys Cys Lys Ala
165 170 175
Glu Asn Ala Val Glu Cys Phe Gln Thr Lys Ala Ala Thr Val Thr Lys
180 185 190
Glu Leu Arg Glu Ser Ser Leu Leu Asn Gln His Ala Cys Ala Val Met
195 200 205
Lys Asn Phe Gly Thr Arg Thr Phe Gln Ala Ile Thr Val Thr Lys Leu
210 215 220
Ser Gln Lys Phe Thr Lys Val Asn Phe Thr Glu Ile Gln Lys Leu Val
225 230 235 240
Leu Asp Val Ala His Val His Glu His Cys Arg Gly Asp Val Leu
245 250 255
Asp Cys Leu Gln Asp Gly Glu Lys Ile Met Ser Tyr Ile Cys Ser Gln
260 265 270
Gln Asp Thr Leu Ser Asn Lys Ile Thr Glu Cys Cys Lys Leu Thr Thr
275 280 285
Leu Glu Arg Gly Gln Cys Ile Ile His Ala Glu Asn Asp Glu Lys Pro
290 295 300
Glu Gly Leu Ser Pro Asn Leu Asn Arg Phe Leu Gly Asp Arg Asp Phe
305 310 315 320
Asn Gln Phe Ser Ser Gly Glu Lys Asn Ile Phe Leu Ala Ser Phe Val
325 330 335
His Glu Tyr Ser Arg Arg His Pro Gln Leu Ala Val Ser Ile Leu
340 345 350
Arg Val Ala Lys Gly Tyr Gln Glu Leu Leu Glu Lys Cys Phe Gln Thr
355 360 365
Glu Asn Pro Leu Glu Cys Gln Asp Lys Gly Glu Glu Glu Leu Gln Lys
370 375 380
Tyr Ile Gln Glu Ser Gln Ala Leu Ala Lys Arg Ser Cys Gly Leu Phe
385 390 395 400
Gln Lys Leu Gly Glu Tyr Tyr Leu Gln Asn Ala Phe Leu Val Ala Tyr
405 410 415
Thr Lys Lys Ala Pro Gln Leu Thr Ser Ser Glu Leu Met Ala Ile Thr
420 425 430
Arg Lys Met Ala Ala Thr Ala Ala Thr Cys Cys Gln Leu Ser Glu Asp
435 440 445
Lys Leu Leu Ala Cys Gly Glu Gly Ala Ala Asp Ile Ile Gly His
450 455 460
Leu Cys Ile Arg His Glu Met Thr Pro Val Asn Pro Gly Val Gly Gln
465 470 475 480
Cys Cys Thr Ser Ser Tyr Ala Asn Arg Arg Pro Cys Phe Ser Ser Leu
485 490 495
Val Val Asp Glu Thr Tyr Val Pro Pro Ala Phe Ser Asp Asp Lys Phe

500 505 510
 Ile Phe His Lys Asp Leu Cys Gln Ala Gln Gly Val Ala Leu Gln Thr
 515 520 525
 Met Lys Gln Glu Phe Leu Ile Asn Leu Val Lys Gln Lys Pro Gln Ile
 530 535 540
 Thr Glu Glu Gln Leu Glu Ala Val Ile Ala Asp Phe Ser Gly Leu Leu
 545 550 555 560
 Glu Lys Cys Cys Gln Gly Gln Glu Gln Glu Val Cys Phe Ala Glu Glu
 565 570 575
 Gly Gln Lys Leu Ile Ser Lys Thr Arg Ala Ala Leu Gly Val
 580 585 590

<210> 3
 <211> 2032
 <212> DNA
 <213> Homo sapiens

<400> 3
 tccatattgt gcttccacca ctgcccaataa caaaataact agcaaccatg aagtgggtgg 60
 aactcaatttt tttaattttt ctactaattt ttactgaatc cagaacactg catagaaatg 120
 aatatggaaat agcttccata ttggattctt accaatgtac tgcagagata agtttagctg 180
 acctgtgtac catatttttt gccagtttg ttcaagaagc cacttacaag gaagttagca 240
 aaatggtgaa agatgcattg actgcaattg agaaaccac tggagatgaa cagtcttcag 300
 ggtgtttaga aaaccagcta cctgcctttc tggagaact ttgcctatg aaagaacttt 360
 tggagaagta cggacattca gactgctgca gccaaagtga agaggggaag cataactgtt 420
 ttcttgca caaaaagccc actccagcat cgtatccact ttccaagt ttccaactgt 480
 tcacaagctg tgaagcatat gaagaagaca gggagacatt catgaacaaa ttctatttat 540
 agatagcaag aaggtcatcc ttctgtatg cactacaat tcttctttgg gctgctctgt 600
 atgacaaaaa aattccatct tgctgcaag ctgaaaaatc agttgaatgc ttccaaacaa 660
 aggcagcaac agttacaaaa gaattaagag aaagcagctt gttaaatcaa catgcatgtg 720
 cagtaaatgaa aaattttggg acccgaaact tccagccat aactgttact aaactgagtc 780
 agaagtttac caaagtttaatt ttactgaata tccagaact agtcttggat gtggccctag 840
 tcatgagca ctgttgcgaa ggagatgtgc tggattgtct gcaggatggg gaaaaataca 900
 ttctctacat atgtttctcaa caagacactc tgtcaacaaa aataacagaa tgcgtcaaac 960
 tgaccacgct ggaacgttgt caatgtataa ttcatgcaga aaatgatgaa aaactggaag 1020
 gctatctccc aaattcaaac aggttttttag gagatagaga tttaaccaa ttttcttcag 1080
 gggaaaaaaa tatcttcttg caagtttttg ttcatgaata ttcaagaaga catctctcagc 1140
 ttgctgtctc agtacttcta agagtgtgcta aaggatacca gtaggttattg gagaagtggt 1200
 tccagactga aaacctctt gaatgccaa ataaaggaga agaagaatta cagaataata 1260
 attacttaca aaatcgcttt ctctgtgctt acacaaagaa agccccccag ctgacctctg 1380
 cggagctgat gcccatcacc agaaaaatgg cagccacagc agccaactgt tgccaactca 1440
 gtgaggagaa actatttgcc tgtggcgagg gagcggtcta cattattatc ggacacttat 1500
 gtatcagaca tgaagtactc ctagtaaacg cctggttttg ccagtgtctc actcttcat 1560
 atgccaaacag gaggccatgc ttccagcagct tgggtgtgga tgaacatat gtccctcctg 1620
 cattctctga tgaacagatc attttccata aggatctgtg ccaagctcag ggtgtagcgc 1680
 tgcacaacgat tgaagctgtc ttctcattat acctgtgaa gcaaaagcca caaataacag 1740
 aggaacaact tgaagctgtc attgcagatt tctcaggct gtggagaaa tgcgtcccaag 1800
 gcaggagaca ggaagtctgc ttgtgtgaag agggacaaaa actgatttca aaaactgtg 1860
 ctgctttggg agtttaaatc acttcagggg aagagaagac aaacagagtc ttcatctcg 1920
 tgtgaacttt tctcttaatt tttaactgat ttaacacttt tgtgaatta atgaatgat 1980
 aaagactttt atgtgagatt tctttatcac agaaataaaa tatctccaaa tg 2032

<210> 4
 <211> 1773
 <212> DNA
 <213> Homo sapiens

```

<400> 4
acactgcata gaaatgaata tggaaatagct tccatatatgg attcttaccac atgtactgca 60
gagataagtt tagctgacct ggctaccata ttttttggcc agttttgttca agaagccact 120
tacaagggaag taagcaaaat ggtgaaagat gcattgactg caattgagaa acccactgga 180
gatgaacagt cttcagggtg tttagaaaac cagctacctg cctttctgga agaactttgc 240
catgagaaag aaattttggga gaagtacgga cattcagact gctgcagcca aagtgaagag 300
ggaagacata actgttttct tgcacacaaa aagcccactc cagcatcgat ccacttttcc 360
caagttccag aacctgtcac aagctgtgaa gcatatgaag aagacagggga gacattcatg 420
aacaatttca tttatgagat agcaagaagg catccttctc tgtatgcacc tacaatttctt 480
ctttgggtct ctgcctatga caaaaataat ccatcttctc gcaagactga aaatgcagtt 540
gaatgcttcc aaacaagggc agcaacagtt acaaaagaat taagagaaaag cagcttgtta 600
aatcaacatg catgtgcagt aatgaaaaat tttgggaccc gaactttcca agccataact 660
gttactaaac tgagtcagaa gtttaccaaa gttaatttta ctgaaatcca gaaactagtc 720
ctggatgtgg cccatgtaca tgagcactgt tgcagaggag atgtgtctgga ttgtctgcag 780
gatggggaaa aaatcatgtc ctacatatgt tctcaacaag acactctgtc aaacaaaata 840
acagaatgct gcaaaactgac cagctgggaa cgtggtcaat gtataattca tgcagaaaat 900
gatgaaaaac ctgaaggtct atctccaaat ctaaacaggt ttttaggaga tagagatttt 960
aaccaatttt ctccaggggga aaaaaatatc ttcttggcaa gttttgttca tgaattttca 1020
agaagacatc ctcagcttgc tgtctcagta attctaagag ttgctaaagg ataccaggag 1080
ttattggaga agtgttttcca gactgaaaac cctcttgaat gccaaagataa aggagaagaa 1140
gaattacaga aatacatcca ggagagccaa gcattggcaa agcgaagctg cggcctcttc 1200
cagaaaactg gagaatatta ctacaaaaat gcgtttctcg ttgcttacac aaagaagaac 1260
ccccagctga cctgtctgga gctgatggcc atcaccagaa aaatggcagc cacagcagcc 1320
actgtttggc aactcagtha ggacaaaacta ttggcctgtg gcgaggggag ggctgacatt 1380
attatcgagc acttatgtat cagacatgaa atgactccag taaaccttgg tgttggccag 1440
tgctgcactt ctctcatatg caacaggagg ccatgttcca gcagcttggg ggttggtgaa 1500
acatgtctcc cctctgcatt ctctgatgac aagttcattt tccataagga tctgtgacaa 1560
gctcagggtg tagcgtctga aacgatgaag caagagtttc tcattaaact tgtgagagaa 1620
aagccacaaa taacagagga acaacttgag gctgtcattg cagatttctc aggcctgttg 1680
gagaaatgct gccaaaggcca ggaacaggaa gtctgctttg ctgaagaggg acaaaaaactg 1740
atttcaaaaa ctctgctgtc tttgggagtt taa 1773

```

```

<210> 5
<211> 19
<212> PRT
<213> Homo sapiens

```

```

<400> 5
Met Lys Trp Val Glu Ser Ile Phe Leu Ile Phe Leu Leu Asn Phe Thr
1 5 10 15
Glu Ser Arg

```

```

<210> 6
<211> 21
<212> DNA
<213> Homo sapiens

```

```

<400> 6
aaactcgaga agtgggtgga a 21

```

```

<210> 7
<211> 24
<212> DNA
<213> Homo sapiens

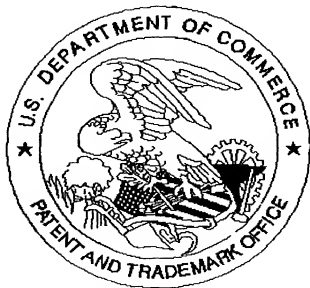
```

```

<400> 7
aaactcgagt taaactccca aagc 24

```

United States Patent & Trademark Office
Office of Initial Patent Examination -- Scanning Division



Application deficiencies found during scanning:

☐ Page(s) _____ of _____ were not present
for scanning. (Document title)

☐ Page(s) _____ of _____ were not present
for scanning. (Document title)

☐ **Scanned copy is best available.**

Declaration pages have lines.